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(54) Title: NOVEL CHEMOKINE FOR MOBILIZING STEM CELLS

(57) Abstract

Novel chemokines for mobilizing stem cells are provided. Methods of mobilizing stem cells are also provided.

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NOVEL CHEMOKINE FOR MOBILIZING STEM CELLS

Background of the Invention

Hematopoietic cells have very important roles in a number of different processes in the body. For example, leukocytic hematopoietic cells are important in maintaining the body's defenses against disease; monocytes, macrophages and lymphocytes are involved in potentiating the body's responses to infection and tumors, while granulocytes are involved in overcoming infection, parasites and tumors. Platelets, another hematopoietic cell, form an important element in the hemostatic mechanism through initiating thrombus formation by their adhesion to each other and to damaged surfaces, and by the release of factors which assist in the formation of the fibrin clot. Erythrocytes are mainly involved in the transport of oxygen.

All of these blood cells are derived from a single progenitor cell called the hematopoietic stem cell. Stem cells are both pluripotent, in that they give rise to all different cell types, and capable of self renewal. Hematopoietic stem cells make up only a small percentage of bone marrow cells and are normally quiescent. However, when stimulated to divide, these stem cells produce a differentiated daughter cell with great proliferative potential. Sequential rounds of division and differentiation give rise to an enormous amplification of cell numbers which is necessary for the production of mature blood cells. This process of division and differentiation is subject to regulation at many levels to control cell production.

Numerous studies have led to the definition of functions of several hematopoietic regulatory messengers. These biomolecules have been characterized as stimulatory, e.g., Colony Stimulating Factors (CSFs) and interleukins (IL-1, IL-3, IL-5 and IL-9); inhibitory, e.g., transforming growth factor-β (TGF-β), interferon, prostaglandin E, tumor necrosis factor, macrophage inflammatory protein-1 (MIP-1), lactoferrin, acidic isoferritins, AcSKDP, and pEEDCK (a synthetic HP5B monomer); or enhancing, e.g., TGF-β, IL-6, IL-4, IL-9, IL-11, MIP-1, MIP-2, leukemia inhibitory factor and *Steel* factor. Pelus et al. *Experimental Hematology* **1994**, 22:239-247. Stimulatory biomolecules have been found to promote division of

particular cell lineages. For example, G-CSF derives neutrophil production, while erythropoietin promotes formation of erythrocytes.

A number of these biomolecules and additional agents have been found to induce the mobilization of hematopoietic stem cells.

A single injection of IL-8 has been shown to induce mobilization of pluripotent stem cells that are able to provide permanent reconstitution of myeloid cells and of T and B lymphocytes. Laterveer et al. *Blood* **1995**, **85**(8):2269-2275. IL-8 belongs to a family of pro-inflammatory molecules called chemokines. This family has been divided into two subfamilies, the CXC and CC chemokines, based on whether the first two cysteine residues in a conserved motif are adjacent to each other or are separated by an intervening residue. In general, CXC, which include IL-8, melanoma growth-stimulating activity (MGSA) and platelet factor 4 (PF4), are potent chemoattractants and activators of neutrophils but not monocytes. In contrast, CC chemokines, which include RANTES, monocyte chemotactic protein 1 (MCP-1) and MIP-1, are chemoattractants for monocytes but not neutrophils.

Stem cell inhibitors (SCIs) such as the CC chemokines, murine and human MIP-1α (LD78), have also been shown to enhance the release and mobilization of cells into the peripheral blood. WO 94/28916; Simm et al. *Blood* **1994**, **84**:2937.

Increased mobilization of stem cells in patients treated with sequentially administered interleukin-3 and GM-CSF compared with GM-CSF alone has been reported by Brugger et al. *Blood* **1992**, **79**:1193-1200. In addition, it has been shown that the absolute number of peripheral blood progenitor cells can be expanded *in vitro* by culture in a cocktail of cytokines, usually including SCF, IL-3, and either IL-6 or IL-1. Bodine, D. *Experimental Hematology* **1995**, **23**:293-295.

SK&F 107647, a hematoregulatory agent containing an ethylene bridge in place of the cysteine bridge of HP5B, has been demonstrated to be a potent stimulator of *in vitro* myelopoiesis. Pelus et al. *Experimental Hematology* **1994**, **22**:239-247. Injection of SK&F 107647 in normal mice resulted in a two- to six-fold increase in serum colony-stimulating activity. Administration of this agent over 4 days resulted in significant increases in the number of granulocyte-macrophage,

erythroid, and multipotential progenitor cells, as well as stimulating their cell cycle rates.

It has also been found that pretreatment with stem cell stimulating factor such as G-CSF can expand the pool of progenitor cells susceptible for mobilization by these agents, further increasing their mobilizing effect. For example, the combination of MIP-1α with G-CSF was found to increase white cell count in the blood as compared to G-CSF alone. Simm et al. *Blood* **1994**, **84**:2937. Co-administration of SCI with G-CSF caused the enhanced mobilization of a number of cell types including neutrophils, monocytes, eosinphils, lymphocytes and basophils. WO 94/28916. Administration of G-CSF alone had no effect on the release of eosinphils or basophils after 2 days of administration. Similar effects were observed when other agents such as GM-CSF, f-MET-Leu-Phe or IL-8 were coadministered with SCIs.

New chemokines have now been identified which also mobilize stem cells in an animal. These chemokines can be administered alone, or in combination with a colony stimulating factor or hemoregulatory agent to enhance mobilization of stem cells.

Summary of the Invention

An object of the present invention is to provide novel chemokines for the mobilization of stem cells in an animal.

Another object of the invention is to provide a method of mobilizing stem cells.

Brief Description of the Drawings

Figure 1 shows the sequence and alignment of the novel chemokines with known chemokines.

Detailed Description of the Invention

In recent years, the availability of recombinant cytokines and the use of hematopoietic stem cell support have resulted in the widespread application of high-dose chemotherapy regimens designed to improve the success of cancer therapy.

Despite significant advances, however, delayed recovery of hematopoiesis remains an important source of morbidity and mortality for patients treated with this approach. Since their discovery over 20 years ago, peripheral blood hematopoietic progenitor cells (PBPCs) have been increasingly used to supplement and even replace bone marrow as the source of hematopoietic support in a variety of situations.

Purified populations of cells are increasingly being used therapeutically and it would therefore be advantageous to be able to increase the number of circulating blood cells. It is useful to be able to harvest hematopoietic cells prior to chemotherapy or radiotherapy, thus, protecting them from harmful effects of this therapy; after therapy, the cells can be returned to the patient. It would therefore be highly beneficial to provide an agent which promoted the release and mobilization of a number of hematopoietic cells. Such an agent would be useful for enhancing the response to infection.

Peripheral blood cell transplantation is an important procedure in the treatment of cancer patients with high dose chemotherapy. In such treatment, patients are treated to induce clinical remission of their cancer, then during the remission, successive treatment with CSF, for example, by priming with cyclophosphamide then administration of G-CSF, causes eventual mobilization of cells from the bone marrow to the peripheral circulation for harvesting of leukophoresed blood; then the patient is given high dose chemotherapy or radiotherapy and the resultant bone marrow failure is compensated for by infusion of the stored blood or cells collected previously. This procedure may be modified by the omission of the initial induction of remission, and whole blood may be collected rather than leukophoresed blood. The mobilization effects of the present invention makes it a candidate both to replace CSFs in such cancer treatment regimes, and also to complement the mobilization effects of CSFs in combined treatments.

The two subfamilies of chemokines (CXC and CC) are ever expanding and presumably the individual members have similar, if slightly divergent, functions. The chemokines disclosed in the present invention are new members of the CC subfamily and are structurally similar to MCP-1, MCP-3, hRANTES, mMIP-1α, and mMIP-1β (Figure 1). The effect of these chemokines in inducing leukophilia will find clinical

and veterinary application in all utilities where the raising of hematopoietic cell levels is important. For example, a chemokine of the present invention can be used to enhance immune responses against chronic infections, particularly parasitic and bacterial infections. It may also have a role in promoting wound healing.

The chemoattractant activity of these chemokines can be boosted by pretreatment with a colony stimulating factor such as G-CSF or GM-CSF. Alternatively, the hematoregulatory peptides SK&F 107647 (currently in clinical trials), FLT-3 ligand (Immunex) or any other G-CSF mimetics (peptide and non-peptide) may be used. These stimulants may have an even more dramatic effect on these novel chemokines than on those already known due to their slight structural differences. For example, CKB-6 in combination with G-CSF was effective as a mobilizing factor. As known in the art, these peptides are useful in stimulating myelopoiesis in patients suffering from reduced myelopoietic activity, including bone marrow damage, agranulocytosis and aplastic anemia. Also included are patients who have depressed bone marrow function due to immunosuppressive treatment to suppress tissue reactions (i.e., bone marrow transplant surgery). They may also be used to promote more rapid regeneration of bone marrow after cytostatic chemotherapy and radiation therapy for neoplastic and viral diseases. There may also be a value where patients have serious infections due to a lack of immune response following bone marrow failure.

The hematopoietic stem cells released and harvested in the manner described above may be useful for subsequent *in vitro* and *ex vivo* manipulations to deliver gene products in gene therapy. Another embodiment is co-administration with cytotoxic drugs.

The following examples are provided for illustrative purposes only and are not intended to limit the invention.

EXAMPLES

Example 1: Mobilization Assay for Novel Chemokines as Single Agents

A panel of novel chemokines will be tested as individual stem cell mobilization agents in BDF 1 mice. These chemokines include, but should not be

limited to: Ckβ-1, Ckβ-4, Ckβ-6, Ckβ-7, Ckβ-8, Ckβ-9, Ckβ-10, Ckβ-11, Ckβ-12, Ckβ-13, and Ckα-1. Each agent will be assayed in concentrations of 50, 10, and 2 μg/mouse and administered via SC, IM, or a PO route. The kinetics of chemokine mobilization of stem cells will be monitored in 15 minute intervals over a period of 60 minutes by collecting blood samples from the mice by cardiac puncture. The mobilized stem cells will be collected by a densing gradient (Lympholyte M). Cells are washed then frozen for future usage. The mobilization profile of the blood differentials will be assessed using a Technicon H1 hematology analyzer. Mobilization of inflammatory cells such as PMN's, eosinophils, and basophils will be taken into account when evaluating the overall potential inflammatory profile. The chemokine IL-8, which mobilizes hematopoietic stem cells as a single factor, will be included in these studies as a positive control.

Example 2: Mobilization Assay for Novel Chemokines in Combination with Hematostimulants

In these studies, hematostimulants will be assayed in combination with the aforementioned chemokines as mobilization factors. These agents include: G-CSF, GM-CSF, SK&F 107647, and FLT-3 ligand. However, any G-CSF mimetic (hematostimulants which are not colony stimulating factors like G-CSF or GMCSF, but have hematopoietic activity) may be used. In combination studies, G-CSF will be administered IP to mice four days prior to the novel chemokines. As in Example 1, the dose of chemokine and time of blood collection will be varied. Combination studies with hematostimulant pre-treatment will utilize MIP-1α as the positive control.

Example 3: CFU Assay

Blood samples collected during the mobilization phase will be assessed for colony forming units (CFU-GM) at days 7 and 14. Cells are adjusted to $2X10^6$ cells/ml in McCoys medium with 15 x FBS serum. A single layer agar system utilizing the following is used: McCoys medium enriched with nutrients ($NaHCO_3$,

pyruvate, amino acids and vitamins); 0.3% Bacto agar. To this is added cells from the blood samples (final concentration = $2X10^5$ cells/ml). The agar plates are incubated at 37°C, 5% CO_2 for 7 days. Colonies of proliferating cells (CFU-GM) are counted utilizing a microscope. In addition, early hematopoietic high proliferative potential (HPP) progenitors, will be counted in the day 14 CFU cultures.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SmithKline Beecham Corporation and Human Genome Sciences, Inc.

(ii) TITLE OF INVENTION: Novel Chemokine for Mobilizing Stem Cells

(iii) NUMBER OF SEQUENCES: 19

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(E) COUNTRY: USA

(F) ZIP: 19406-0939

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
THR LYS THR GLU SER SER SER ARG GLY PRO TYR HIS PRO SER GLU
1               5                  10                  15
CYS CYS PHE THR TYR THR THR TYR LYS ILE PRO ARG GLN ARG ILE
                20                  25                  30
MET ASP TYR TYR GLU THR ASN SER GLN CYS SER LYS PRO GLY ILE
                35                  40                  45
VAL PHE ILE THR XAA ARG GLY HIS SER VAL CYS THR ASN PRO SER
                50                  55                  60
ASP LYS TRP VAL GLN ASP TYR ILE LYS ASP MET LYS
                65                  70
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
ALA SER ASN PHE ASP CYS CYS LEU GLY TYR THR ASP ARG ILE LEU
1               5                  10                  15
HIS PRO LYS PHE ILE VAL GLY PHE THR ARG GLN LEU ALA ASN ASX
                20                  25                  30
GLY CYS ASP ILE ASN ALA ILE ILE PHE HIS THR LYS LYS LYS LEU
                35                  40                  45
SER VAL CYS ALA ASN PRO LYS GLN THR TRP VAL LYS TYR ILE VAL
                50                  55                  60
ARG LEU LEU SER LYS LYS VAL LYS ASN MET
                65                  70
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
VAL VAL ILE PRO SER PRO CYS CYS MET PHE PHE VAL SER LYS ARG
1               5                  10                  15
ILE PRO GLU ASN ARG VAL VAL SER TYR GLN LEU SER SER ARG SER
            20                  25                  30
THR CYS LEU LYS GLY GLY VAL ILE PHE THR THR LYS LYS GLY GLN
            35                  40                  45
GLN PHE CYS GLY ASP PRO LYS GLN GLU TRP VAL GLN ARG TYR MET
            50                  55                  60
LYS ASN LEU ASP ALA LYS GLN LYS LYS ALA
            65                  70
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
ALA GLN VAL GLY THR ASN LYS GLU LEU CYS CYS LEU VAL TYR THR
1               5                  10                  15
SER TRP GLN ILE PRO GLN LYS PHE ILE VAL ASP TYR SER GLU THR
            20                  25                  30
SER PRO GLN CYS PRO LYS PRO GLY VAL ILE LEU LEU THR LYS ARG
            35                  40                  45
GLY ARG GLN ILE CYS ALA ASP PRO ASN LYS LYS TRP VAL GLN LYS
            50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
GLU ASN PRO VAL LEU LEU ASP ARG PHE HIS ALA THR SER ALA ASP
1               5                  10                  15
CYS CYS ILE SER TYR THR PRO ARG SER ILE PRO CYS SER LEU LEU
            20                  25                  30
```

GLU SER TYR PHE GLU THR ASN SER GLU CYS SER LYS PRO GLY VAL
35 40 45
ILE PHE LEU THR LYS LYS GLY ARG ARG PHE CYS ALA ASN PRO SER
50 55 60
ASP LYS GLN VAL GLN VAL CYS MET ARG MET LEU LYS LEU ASP THR
65 70 75
ARG ILE LYS THR ARG LYS ASN
80

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

SER ASP ALA GLY GLY ALA GLN ASP CYS CYS LEU LYS TYR SER GLN
1 5 10 15
ARG LYS ILE PRO ALA LYS VAL VAL ARG SER TYR ARG LYS GLN GLU
20 25 30
PRO SER LEU GLY CYS SER ILE PRO ALA ILE LEU PHE LEU PRO ARG
35 40 45
LYS ARG SER GLN ALA GLU LEU CYS ALA ASP PRO LYS GLU LEU TRP
50 55 60
VAL GLN GLN LEU MET GLN HIS LEU ASP LYS THR PRO SER PRO GLN
65 70 75
LYS PRO ALA GLN

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

PHE ASN PRO GLN GLY LEU ALA GLN PRO ASP ALA LEU ASN VAL PRO
1 5 10 15
SER THR CYS CYS PHE THR PHE SER SER LYS LYS ILE SER LEU GLN
20 25 30
ARG LEU LYS SER TYR VAL ILE THR THR SER ARG CYS PRO GLN LYS
35 40 45

```
ALA VAL ILE PHE ARG THR LYS LEU GLY LYS GLU ILE CYS ALA ASP
                50                  55                  60
PRO LYS GLU LYS TRP VAL GLN ASN TYR MET LYS HIS LEU GLY ARG
                65                  70                  75
LYS ALA HIS THR LEU LYS THR
                80
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 83

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
PRO ALA PRO THR LEU SER GLY THR ASN ASP ALA GLU ASP CYS CYS
1               5                  10                  15
LEU SER VAL THR GLN LYS PRO ILE PRO GLY TYR ILE VAL ARG ASN
                20                  25                  30
PHE HIS TYR LEU LEU ILE LYS ASP GLY CYS ARG VAL PRO ALA VAL
                35                  40                  45
VAL PHE THR THR LEU ARG GLY ARG GLN LEU CYS ALA PRO PRO ASP
                50                  55                  60
GLN PRO TRP VAL GLU ARG ILE ILE GLN ARG LEU GLN ARG THR SER
                65                  70                  75
ALA LYS MET LYS ARG ARG SER SER
                80
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
ARG SER GLN PRO LYS VAL PRO GLU TRP VAL ASN THR PRO SER THR
1               5                  10                  15
CYS CYS LEU LYS TYR TYR GLU LYS VAL LEU PRO ARG ARG LEU VAL
                20                  25                  30
VAL GLY TYR ARG LYS ALA LEU ASN CYS HIS LEU PRO ALA ILE ILE
                35                  40                  45
```

```
PHE VAL THR LYS ARG ASN ARG GLU VAL CYS THR ASN PRO ASN ASP
                50                  55                  60
ASP TRP VAL GLN GLU TYR ILE LYS ASP PRO ASN LEU PRO LEU LEU
                65                  70                  75
PRO THR ARG ASN LEU SER THR
                80
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
PRO TYR GLY ALA ASN MET GLU ASP SER VAL CYS CYS ARG ASP TYR
1               5                  10                  15
VAL ARG TYR ARG LEU PRO LEU ARG VAL VAL LYS HIS PHE TYR TRP
                20                  25                  30
THR SER ASP SER CYS PRO ARG PRO GLY VAL VAL LEU LEU THR PHE
                35                  40                  45
ARG ASP LYS GLU ILE CYS ALA ASP PRO ARG VAL PRO TRP VAL LYS
                50                  55                  60
MET ILE LEU ASN LYS LEU SER GLN
                65
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
ALA SER PRO TYR SER SER ASP THR THR PRO CYS CYS PHE ALA TYR
1               5                  10                  15
ILE ALA ARG PRO LEU PRO ARG ALA HIS ILE LYS GLU TYR PHE TYR
                20                  25                  30
```

THR SER GLY LYS CYS SER ASN PRO ALA VAL VAL PHE VAL THR ARG
35 40 45
LYS ASN ARG GLN VAL CYS ALA ASN PRO GLU LYS LYS TRP VAL ARG
50 55 60
GLU TYR ILE ASN SER LEU GLU MET SER
65

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ALA PRO TYR GLY ALA ASP THR PRO THR ALA CYS CYS PHE SER TYR
1 5 10 15
SER ARG LYS ILE PRO ARG GLN PHE ILE VAL ASP TYR PHE GLU THR
20 25 30
SER SER LEU CYS SER GLN PRO GLY VAL ILE PHE LEU THR LYS ARG
35 40 45
ASN ARG GLN ILE CYS ALA ASP SER LYS GLU THR TRP VAL GLN GLU
50 55 60
TYR ILE THR ASP LEU GLU LEU ASN ALA
65

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
ALA PRO MET GLY SER ASP PRO PRO THR SER CYS CYS PHE SER TYR
1               5                  10                  15
THR SER ARG GLN LEU HIS ARG SER PHE VAL MET ASP TYR TYR GLU
                20                  25                  30
THR SER SER LEU CYS SER LYS PRO ALA VAL VAL PHE LEU THR LYS
                35                  40                  45
ARG GLY ARG GLN ILE CYS ALA ASN PRO SER GLU PRO TRP VAL THR
                50                  55                  60
GLU TYR MET SER ASP LEU GLU LEU ASN
                65
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 78
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
LEU ALA GLN PRO ASP ALA ILE ASN ALA PRO VAL THR CYS CYS TYR
1               5                  10                  15
ASN PHE THR ASN ARG LYS ILE SER VAL GLN ARG LEU ALA SER TYR
                20                  25                  30
ARG ARG ILE THR SER SER LYS CYS PRO LYS GLU ALA VAL ILE PHE
                35                  40                  45
LYS THR ILE VAL ALA LYS GLU ILE CYS ALA ASP PRO LYS GLN LYS
                50                  55                  60
TRP VAL GLN ASP SER MET ASP HIS LEU ASP LYS GLN THR GLN THR
                65                  70                  75
PRO LYS THR
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
SER PRO GLN GLY LEU ALA GLN PRO VAL GLY ILE ASN THR SER THR
1               5                  10                  15
THR CYS CYS TYR ARG PHE ILE ASN LYS LYS ILE PRO LYS GLN ARG
            20                  25                  30
LEU GLU SER TYR ARG ARG THR THR SER SER HIS CYS PRO ARG GLU
        35                  40                  45
ALA VAL ILE PHE LYS THR LYS LEU ASP LYS GLU ILE CYS ALA ASP
    50                  55                  60
PRO THR GLN LYS TRP VAL GLN ASP PHE MET LYS HIS LEU ASP LYS
65                  70                  75
LYS THR GLN THR PRO LYS LEU
            80
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
SER ALA LYS GLU LEU ARG CYS GLN CYS ILE LYS THR TYR SER LYS
1               5                  10                  15
PRO PHE HIS PRO LYS PHE ILE LYS GLU LEU ARG VAL ILE GLU SER
            20                  25                  30
GLY PRO HIS CYS ALA ASN THR GLU ILE ILE VAL LYS LEU SER ASP
        35                  40                  45
GLY ARG GLU LEU CYS LEU ASP PRO LYS GLU ASN TRP VAL GLN ARG
    50                  55                  60
VAL VAL GLU LYS PHE LEU LYS ARG ALA GLU ASN SER
65                  70
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
ALA GLU LEU ARG CYS MET CYS ILE LYS THR THR SER GLY ILE HIS
1               5                  10                  15
PRO LYS ASN ILE GLN SER LEU GLU VAL VAL ILE GLY LYS GLY THR
            20                  25                  30
HIS CYS ASN GLN VAL GLU VAL ILE ALA THR LEU LYS ASP GLY ARG
            35                  40                  45
LYS ILE CYS LEU ASP PRO ASP ALA PRO ARG ILE LYS LYS ILE VAL
            50                  55                  60
GLN LYS LYS LEU ALA GLY ASP GLU SER ALA ASP
            65                  70
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
GLU ALA GLU GLU ASP GLY ASP LEU GLN CYS LEU CYS VAL LYS THR
1               5                  10                  15
THR SER GLN VAL ARG PRO ARG HIS ILE THR SER LEU GLU VAL ILE
            20                  25                  30
LYS ALA GLY PRO HIS CYS PRO THR ALA GLN LEU ILE ALA THR LEU
            35                  40                  45
```

LYS ASN GLY ARG LYS ILE CYS LEU ASP LEU GLN ALA PRO LEU TYR
50 55 60
LYS LYS ILE LEU LYS LYS LEU GLU SER
65

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

VAL LEU GLU VAL TYR TYR THR SER LEU ARG CYS ARG CYS VAL GLN
1 5 10 15
GLU SER SER VAL PHE ILE PRO ARG ARG PHE ILE ASP ARG ILE GLN
20 25 30
ILE LEU PRO ARG GLY ASN GLY CYS PRO ARG LYS GLU ILE ILE VAL
35 40 45
TRP LYS LYS ASN LYS SER ILE VAL CYS VAL ASP PRO GLN ALA GLU
50 55 60
TRP ILE GLN ARG MET MET GLU VAL LEU ARG LYS ARG
65 70

What is claimed is:

1. A chemokine comprising SEQ ID NO: 1 capable of mobilizing stem cells.
2. A chemokine comprising SEQ ID NO: 2 capable of mobilizing stem cells.
3. A chemokine comprising SEQ ID NO: 3 capable of mobilizing stem cells.
4. A chemokine comprising SEQ ID NO: 4 capable of mobilizing stem cells.
5. A chemokine comprising SEQ ID NO: 5 capable of mobilizing stem cells.
6. A chemokine comprising SEQ ID NO: 6 capable of mobilizing stem cells.
7. A chemokine comprising SEQ ID NO: 7 capable of mobilizing stem cells.
8. A chemokine comprising SEQ ID NO: 8 capable of mobilizing stem cells.
9. A chemokine comprising SEQ ID NO: 9 capable of mobilizing stem cells.
10. A chemokine comprising SEQ ID NO: 10 capable of mobilizing stem cells.
11. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 1.
12. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 2.
13. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 3.
14. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 4.
15. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 5.
16. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 6.
17. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 7.
18. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 8.
19. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 9.

20. A method of mobilizing stem cells in an animal comprising administering to an animal an effective amount of a chemokine comprising SEQ ID NO: 10.

21. The method of claims 11-20 further comprising administering a colony stimulating factor.

22. The method of claims 11-20 further comprising administering a hematoregulatory agent.

```
hRANTES        ASPYSSDT TPCCFAYIARPLPRAHIKEYFYTSGK  CSNPAVVFVTRKNRQV  CANPEKKWVREYINSLEMS
mMIP-1α         APYGADTPTACCFSY SRKIPRQFIVDYFETSSL  CSQPGVIFLTKRNRQI  CADSKETWVQEYITDLELNA
mMIP-1β         APMGSDPPTSCCFSYTSRQLHRSFVMDYYETSSL  CSKPAVVFLTKRGRQI  CANPSEPWVTEYMSDLELN
MCP-1        LAQPDAINAPVTCCYNFTNRKISVQRLASYRRITSSK CPKEAVIFKTIVAKEI  CADPKQKWVQDSMDHLDKQTQTPKT
MCP-3     SPQGLAQPVGINTSTTCCYRFINKKIPKQRLESYRRTTSSH CPREAVIFKTKLDKEI  CADPTQKWVQDFMKHLDKKTQTPKL
CKβ -1     IKTESSSRGPYHPSECCFTYTTYKIPRQRIMDYYETNSQ  CSKPGIVFITKRGHSV  CTNPSDKWVQDYIKDMK
CKβ -4              ASNFDCCLGYTDRILHPKFIVGFTRQLANEGCDINAIIFHTKKKLSV  CANPKQTWVKYIVRLLSKKVKNM
CKβ -6            VVIPSPCCMFFVSKRIPENRVVSYQLSSRST CLKGGVIFTTKKGQQF  CGDPKQEWVQRYMKNLDAKQKKA
CKβ -7          AQVGTNKELCCLVYTSWQIPQKFIVDYSETSPQ  CPKPGVILLTKRGRQI  CADPNKKWVQK
CKβ -8     ENPVLLDRFHATSADCCISYTPRSIPCSLLESYFETNSE  CSKPGVIFLTKKGRRF  CANPSDKQVQVCMRMLKLDTRIKTRKN
CKβ -9           SDAGGAQDCCLKYSQRKIPAKVVRSYRKQEPSLGCSIPAILFLPRKRSQAELCADPKELWVQQLMQHLDKTPSPQKPAQ
CKβ -10 ENPQGLAQPDALNVPSTCCFTFSSKKISLQRLKSYVITTSR  CPQKAVIFRTKLGKEI  CADPKEKWVQNYMKHLGRKAHTLKT
CKβ -11     PAPTLSGTNDAEDCCLSVTQKPIPGYIVRNFHYLLIKDGCRVPAVVFTTLRGRQL  CAPPDQPWVERIIQRLQRTSAKMKRRSS
CKβ -12   RSQPKVPEWVNTPSTCCLKYYEKVLPRRLVVGYRKALN   CHLPAIIFVTKRNREV  CTNPNDDWVQEYIKDPNLPLLPTRNLST
CKβ -13         PYGANMEDSVCCRDYVRYRLPLRVVKHFYWTSDS  CPRPGVVLLTFRDKEI  CADPRVPWVKMILNKLSQ

NAP-1/IL-8        SAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQRVVEKFLKRAENS
NAP-2               AELRCMCIKTTSGIHPKNIQSLEVVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD
hPF4             EAEEDGDLQCLCVKTTSQVRPRHITSLEVIKAGPH CPTAQLIATLKNGRKICLDLQAPLYKKILKKLES
CKα -1         VLEVYYISLRCRCVQESSVFIPRRFIDRIQILPRGNGCPRKEIIVWKKNKSIVCVDPQAEWIQRMMEVLRKR
```

Substitute Sheet (Rule 26)

FIG. 1

INTERNATIONAL SEARCH REPORT

.tional application No.
PCT/US96/16959

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/52; A61K 38/19
US CL :530/434; 424/85.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/434; 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: chemokine, mip

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	WO 95 18228 A1 (FORSSMANN) 06 July 1995 (06.07.95) Claim 1, SEQ ID NO:6	1,11 --- 21,22 --- 2-10,12-20
X -- Y -- A	WO 95/17092 A1 (HUMAN GENOME SCIENCES, INC.) 29 June 1995 (29.06.95) Claims 10, 12 and 48, Figures 1, 2 and 8.	1,4,5,11, 14 ,15 --- 21,22 --- 2 , 3 , 6 - 10,12,13,16-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 DECEMBER 1996	03 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LORRAINE M. SPECTOR Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

	tional application No. PCT/US96/16959

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Brugger et al. Mobilization of peripheral blood progenitor cells by sequential administration of Interleukin-3 and granulocyte-macrophage colony-stimulating factor following polychemotherapy with etoposide, ifosfamide, and cisplatin. Blood. 01 March 1992, Vol. 79, No. 5, pages 1193-1200, see entire document.	21-22
A	Horuk. R. Molecular properties of the chemokine receptor family. TiPS. May 1994, Vol. 15, pages 159-165.	1-22
Y -- A	Laterveer et al. Interleukin-8 induces rapid mobilization of hematopoietic stem cells with radioprotective capacity and long-term myelolymphoid repopulating ability. Blood. 15 April 1995, Vol. 85, No. 8, pages 2269-2275, see entire document.	21-22 ----- 1-20